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INTRACELLULAR SUPPLIES OF GUANINE RIBONUCLEOTIDES REGULATE THE RESPONSIVENESS OF HUMAN MYELOID PROGENITORS TO GM-CSF

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ABSTRACT We have shown previously that induced maturation of the human myeloid leukemia cell line, HL-60, is associated with a down-regulation of guanine ribonucleotide (G-NTD) synthesis from inosine monophosphate (IMP) and with depletion of GTP and GDP pools. We have also shown that inhibitors of IMP-dehydrogenase (e.g. tiazofurin and mycophenolic acid), which block G-NTD synthesis from IMP, are potent inducers of maturation, while exogenous guanosine, which can be salvaged for G-NTD synthesis by-passing the major synthetic pathway from IMP, prevents induced maturation and promotes cell proliferation to the extent that high G-NTD levels are maintained. These findings prompted us to examine the possibility that guanylate depletion and repletion may affect a GTP-regulated signal transduction pathway for myelopoietic growth factors. As has also been observed by others, we find that recombinant human GM-CSF stimulates the proliferation of HL-60 cells in serum-free suspension culture and in clonal assays. Furthermore, we find that these effects are enhanced by guanosine (10.0 μM) but blocked by the IMP-dehydrogenase inhibitor, tiazofurin (1.0 μM). At concentrations of 1.0 - 5.0 nM, rhGM-CSF may also be shown to stimulate phospholipase C mediated hydrolysis of PIP_2 and to cause rapid, transient increases in cytosolic free Ca^{2+} in HL-60 cells. These effects are also blocked by pretreatment of cells with tiazofurin. We conclude from these studies that intracellular G-NTD supplies may influence the growth and maturation of primitive myeloid progenitors in part by affecting early, GTP-regulated steps in signal-response coupling for myelopoietic growth factors.

INTRODUCTION

A number of human myeloid leukemia cell lines have been established which can not only be maintained as immature, actively dividing blast cells in suspension culture but can also be induced to undergo a form of terminal differentiation in vitro whereby the cells acquire morphologic and functional characteristics of mature neutrophils.[1] These cell lines have attracted considerable interest, for they provide experimentally accessible model systems with which to define basic mechanisms involved in the regulation of myeloid progenitor cell proliferation and differentiation. We have used the HL-60 cell line,[2,3] as well as several other human myeloid lines established in our laboratory,[4,5] to define a role for intracellular guanine ribonucleotides in the regulation of myeloid cell maturation.

In studies reported previously, we have shown that induced myeloid maturation is associated with a depletion of guanosine triphosphate (GTP) and guanosine diphosphate (GDP), while adenylate pools remain relatively intact.[6] This selective depletion of guanylates occurs in part because guanylate synthesis from the central intermediate, inosine monophosphate (IMP), is down-regulated during induced maturation at the rate-limiting step catalyzed by IMP dehydrogenase (IMPD).[4,6-8] We have also shown that specific inhibitors of IMPD such as tiazofurin are potent inducers of maturation in myeloid cell lines.[6,7] Finally, we have shown that the induced myeloid maturation of HL-60 cells is prevented or impaired if intracellular concentrations of guanine ribonucleotides are maintained at high levels. HL-60 cells can utilize exogenous guanine or guanosine to maintain GTP and GDP pools through a salvage pathway that bypasses guanylate synthesis from IMP, and incubation of these cells with guanine or guanosine (1.0 μM to 0.1 mM) prevents both the depletion of guanine ribonucleotides and the induction of myeloid maturation caused by the IMPD inhibitor, tiazofurin.[8] This series of findings, summarized in Figure 1, has led us to conclude that controls of myeloid progenitor cell proliferation and maturation involve a guanine ribonucleotide dependent regulatory system.

In this report we describe studies in which we have examined the possibility that guanine ribonucleotide repletion and depletion might affect a GTP-regulated signal transduction pathway for myelopoietic growth factors. We have addressed this possibility by studying both functional

and biochemical responses of HL-60 cells to high specific activity, human recombinant GM-CSF.

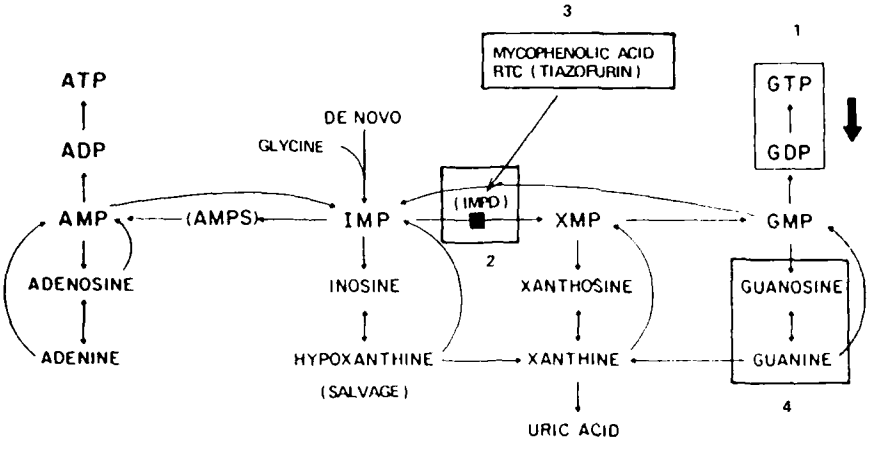

FIGURE 1. (1) Induced maturation of myeloid leukemia cell lines is associated with a depletion of GTP and GDP pools. (2) This depletion of guanine ribonucleotides results at least in part from a down-regulation of guanylate synthesis from IMP at the initial rate limiting step mediated by IMP-dehydrogenase. (3) Specific inhibitors of IMPD are potent inducers of myeloid maturation, but (4) incubation of cells with guanine or guanosine (1.0 μM - 0.1 mM) prevents both guanylate depletion and induction of maturation by these agents.

EXPERIMENTAL APPROACH AND METHODS

In initial studies, we examined the proliferative responses of HL-60 cells to rhGM-CSF, kindly provided to us by Dr.Gordon Wong at Genetics Institute, Cambridge, MA (97.2% pure; specific activity = 4.7×10^6 u/mg). Cell proliferation with and without rhGM-CSF was evaluated both by cell counts in suspension culture and by measurements of clonal growth and plating efficiency of cells in semi-solid media (0.8% methylcellulose).

Since other growth factors [e.g. epidermal growth factor (EGF) and platelet derived growth factor (PDGF)[9,10]] have been found to interact with target cells via a receptor

mediated signal transduction pathway that involves the G-regulated activation of phospholipase C (PLC)[outined in Figure 2], we measured inositol phosphate generation in HL-60 cells with and without added rhGM-CSF under serum-free conditions similar to those in which proliferative responses to GM-CSF could be readily shown. PLC mediated hydrolysis of PIP_2 in lithium chloride treated cells (preincubated with 2-[^{3}H]$_1$myo-inositol) was detected by the methods of Berridge et al.[11] Both the cumulative production of inositol phosphates after 30 min. incubation with rhGM-CSF and the specific production of IP_3 at early time points (15 sec. to 3 min.) were measured. Changes in cytosolic free Ca^{2+} following exposure of cells to rhGM-CSF were also evaluated by measuring Quin 2 fluorescence in cells that had been pre-loaded with 10 μM Quin 2-acetoxymethyl ester.[12]

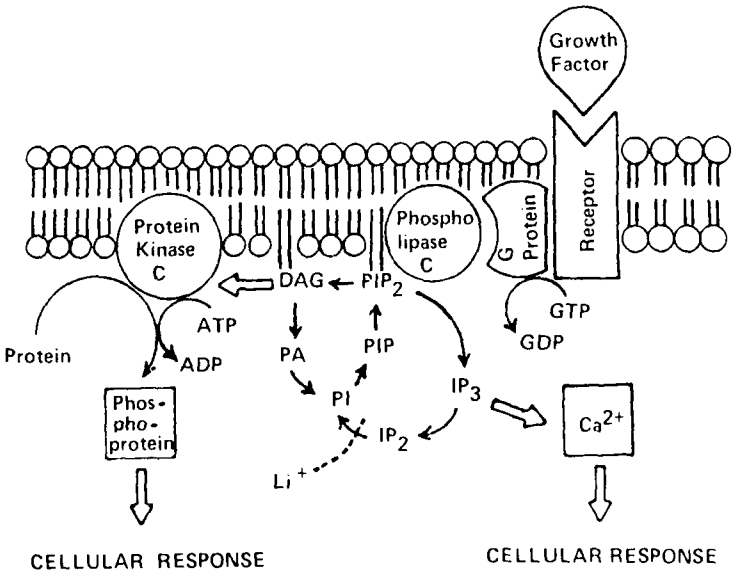

FIGURE 2. Proposed signal transduction pathway for growth factors.[10]

RESULTS

Recombinant, human GM-CSF was found to stimulate the growth of HL-60 cells in suspension culture (Figure 3) and

to increase their plating efficiency in clonal assays by >70%. The rate of clonal expansion was also increased such that colonies formed by cells incubated with rhGM-CSF (0.1 - 1.0 nM) were much larger than those formed spontaneously by cells in the absence of added growth factor. As is illustrated in Figure 3, the addition of guanosine (10.0 μM) to cell cultures substantially enhanced the stimulatory effects of rhGM-CSF on HL-60 cell growth, while tiazofurin (1.0 μM) ablated these effects of CSF.

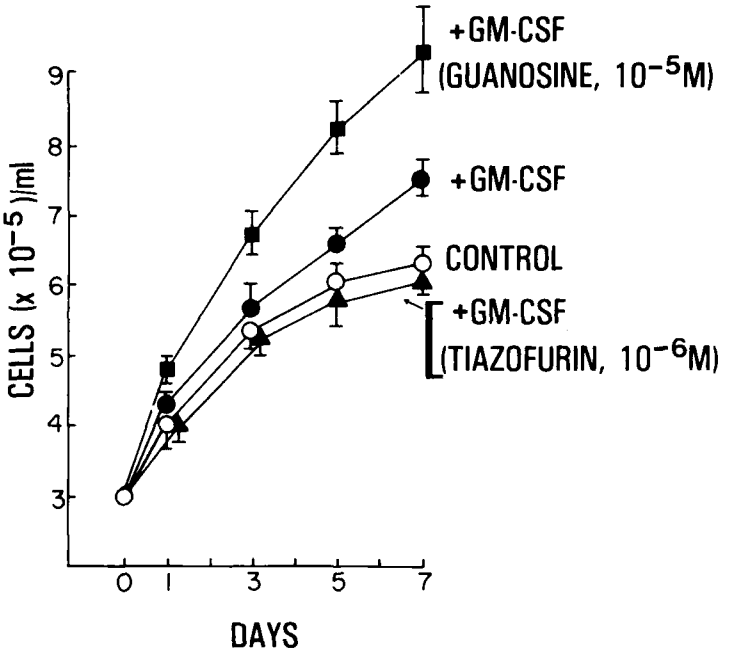

FIGURE 3. Growth of HL-60 cells in suspension culture.

At concentrations of 1.0 to 5.0 nM, rhGM-CSF could also be shown to stimulate PIP_2 hydrolysis and a rapid production of IP_3, as well as rapid, transient increases in cytosolic free Ca^{2+} in HL-60 cells maintained under serum-free culture conditions[6] (Figures 4A and 4B). Moreover, these effects were ablated by preincubation of the cells with tiazofurin (1.0 μM) under conditions shown previously to result in a substantial depletion of GTP pools[6,8] (data not shown).

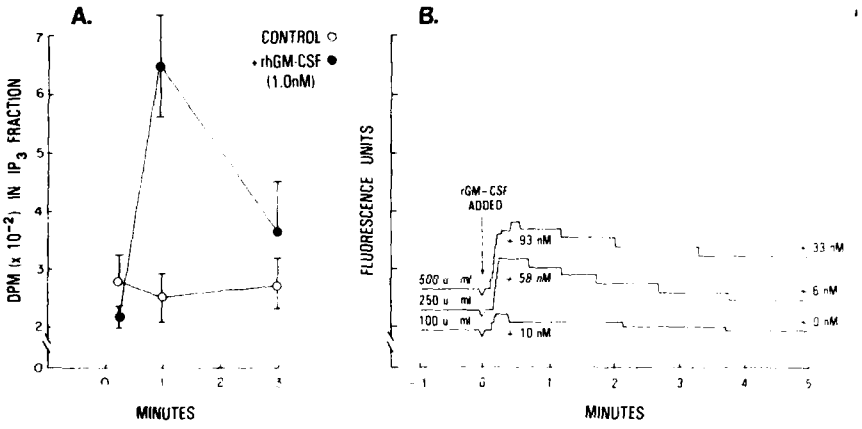

FIGURE 4. Stimulation of IP_3 production (A) and rapid increases in cytosolic free $[Ca^{2+}]$ (B) in HL-60 cells by rhGM-CSF. In panel B the calculated maximum and residual (5 min. following the addition of stimulus) increases in $[Ca^{2+}]_i$ are indicated for the representative fluorescence tracings that are shown.

SUMMARY AND CONCLUSIONS

In the studies described briefly here, we have found that recombinant, human GM-CSF stimulates PIP_2 hydrolysis, production of IP_3, and rapid, transient increases in cytolsolic free Ca^{2+} in HL-60 cells under conditions with which GM-CSF may also be shown to stimulate cellular proliferation. Moreover, these effects of GM-CSF are modulated by pharmacologic manipulations that affect intracellular supplies of guanine ribonucleotides. As we have shown previously, treatment of HL-60 cells with the IMP-dehydrogenase inhibitor, tiazofurin (1.0 μM), causes a substantial depletion of intracellular GTP and GDP pools, inhibits the spontaneous growth of these cells and induces myeloid maturation.[6,8] On the other hand, incubation of the cells with exogenous guanosine (0.01 - 0.1 mM) allows the cells to maintain high guanylate levels, promotes cell proliferation, and inhibits induced maturation.[8] As reported here, guanosine also enhances the stimulatory effects of rhGM-CSF on HL-60 cell growth, while tiazofurin ablates these effects. Moreover, pretreatment of HL-60 cells with

tiazofurin prevents or impairs the stimulatory effects of GM-CSF on PIP_2 hydrolysis and intracellular Ca^{2+} mobilization, while guanosine enhances these effects.

Hence, these studies suggest that intracellular supplies of guanine ribonucleotides may influence the growth and maturation of primitive myeloid cells by affecting a GTP-regulated, phospholipase C mediated signal transduction pathway for myelopoietic growth factors. The independent effects of tiazofurin and guanosine on HL-60 growth in the absence of exogenous growth factors are not inconsistent with this conclusion, for the autonomous growth of these cells has been attributed at least in part to the production of an autocrine growth factor[13,14], and the proliferative effects of this autocrine factor may be mediated via the same signal transduction pathway(s) by which GM-CSF promotes the growth of HL-60 cells.

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